Scientific paper

Is hogfish *Lachnolaimus maximus* (Labridae) a diandric species?

by

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Abstract. – Hogfish *Lachnolaimus maximus* (Walbaum, 1792) is the largest labrid in the Atlantic and a commercially important fish. In previous research, this fish has been defined as a monandric protogynous hermaphrodite, although characterization of hermaphroditism is particularly challenging in labrids. Examination of testicle structure is not always a wholly trustworthy method for distinguishing between diandric species (those with primary and secondary males) and monandric species (only secondary males). In some diandric species, morphological differences in the testes do not occur between primary and secondary males despite functional diandry. The presence of individuals with inactive bisexual gonads and/or of inactive males smaller than the minimum size of sexual maturation is a more dependable criterion for identifying diandry. A histological analysis of the gonads of 1,975 *L. maximus* specimens (10.3-47.4 cm fork length, FL) from the southern Gulf of Mexico was done. Some individuals were identified that were larger than the size at first maturity (N = 20; 24.8-33.5 cm FL) and had ovotestes atypical of a monandric species. These ovotestes had an internal morphology like ovaries, consisting mostly of inactive ovary tissue in lamellar structure, as well as limited development of active testicular tissue in crypts inside the gonad wall and/or in lobules beneath the gonad wall. Considered to be "bisexual" gonads, their origin and function were evaluated based on published data. *Lachnolaimus maximus* could be defined as a diandric species if males can differentiate indirectly by sexual inversion of functional females (postmaturational secondary males) or by direct development from the "bisexual" stage (prematurational secondary males). However, no inactive bisexual individuals or inactive males smaller than the size at first maturity were observed. In addition, population structure and overall sex ratio were typical of a monandric protogynous species. The possibility that *L. maximus* could exhibit diandric hermaphroditism therefore remains hypothetical.

Résumé. – Le labre capitaine *Lachnolaimus maximus* (Labridae) est-il une espèce diandrique ?

Le labre capitaine *Lachnolaimus maximus* (Walbaum, 1792) est le plus grand labridé de l'Atlantique et une espèce d'importance commerciale. Au cours d'études antérieures, ce poisson a été considéré comme une espèce hermaphrodite protogyne monandrique, bien que la caractérisation de l'hermaphrodisme chez les labridés soit particulièrement ardue. L'examen de la structure des testicules ne constitue pas une méthode avérée sûre pour distinguer les espèces diandriques (avec des mâles primaires et secondaires) des espèces monandriques (avec seulement des mâles secondaires). Chez certaines espèces présentant une diandrie fonctionnelle, des différences dans la morphologie des testicules n'apparaissent pas entre mâles primaires et secondaires. La présence d'individus dont les gonades sont bisexuées et/ou de mâles inactifs plus petits que la taille de première maturité représente un critère plus fiable pour l'identification de la diandrie. Dans cette étude, l'analyse histologique des gonades de 1975 spécimens de *L. maximus* (10.3-47.4 cm de longueur à la fourche, LF) du sud du golfe du Mexique a été réalisée. Quelques individus de tailles supérieures à celle de première maturité (N = 20; 24,8-33,5 cm LF) présentant des ovotestis atypiques pour une espèce monandrique furent identifiés. Ces ovotestis, à morphologie interne identique à celle des ovaires, étaient composés principalement de tissu ovarien inactif présent dans une structure lamellaire, mais aussi de tissu testiculaire actif peu développé dans des cryptes situées dans la membrane des gonades et/ou dans des lobules présents en dessous de celle-ci. L'origine et la fonction de ces ovotestis considérés comme des gonades "bisexuées" ont été évaluées sur la base des données disponibles dans la littérature. *Lachnolaimus maximus* pourrait être considéré comme une espèce diandrique si les mâles peuvent se différencier indirectement par l'inversion du sexe chez des femelles fonctionnelles (mâles secondaires postmatures) ou bien par développement direct depuis le stade "bisexuel" (mâles secondaires prématures). Toutefois, aucun individu bisexué inactif ou mâle inactif de taille inférieure à celle de première maturité n'a été observé. De plus, la structure de la population et le sex-ratio furent typiques d'une espèce protogyne monandrique. La possibilité que *L. maximus* puisse présenter un hermaphrodisme diandrique demeure par conséquent hypothétique.

Protogynous sequential hermaphroditic fish generally change sex during their life cycle, passing from a functional female to a functional male phase. Males produced by sexual inversion from functional females are termed secondary males. In some species, however, some males can develop directly from the larval or immature phase without passing through the functional female phase; these are termed primary males. If all males in a species derive from functional

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females via sexual inversion, then it is a monandric species (*i.e.* only one way for males to develop). If some individuals directly differentiate as primary males and these coexist with secondary males, the species is diandric (*i.e.* two ways for males to develop) (Sadovy and Shapiro, 1987; Sadovy de Mitcheson and Liu, 2008). These two types of males were initially identified in species of the family Labridae (wrasses and closely-related parrotfishes; Westneat and Alfaro, 2005), in relation to sexual dimorphism and dichromatism, and mating behaviour (Reinboth, 1962; Choat and Robertson, 1975; Robertson and Warner, 1978; Warner and Robertson, 1978).

Three main germinal tissue configurations can be identified in sequential hermaphrodites: delimited type (a connective tissue boundary separates male from female tissues); undelimited type 1 (male and female tissues are not divided by a connective tissue boundary but never intermix); and undelimited type 2 (male and female tissues are intermixed) (Sadovy and Shapiro, 1987). Consequently, in protogynous species with undelimited gonads, the testes of secondary males retain the internal structure of the ovaries from the previous female phase (*i.e.* lumen and internal lamellar structure) and develop a peripheral sperm duct (sperm sinuses) inside the gonad membrane. By contrast, the testes of secondary males in species with delimited gonads generally do not contain remnants of ovarian forms and develop ducts completely separate from the ovary and its oviduct. Since most labrids exhibit undelimited type 2 germinal tissues (Sadovy and Shapiro, 1987; Sadovy de Mitcheson and Liu, 2008), the internal structural differences between the testes of primary and secondary males are considered the most trustworthy criterion for differentiating between monandry and diandry in this family. Testis morphology in primary males is similar to that of males in gonochoristic species (*i.e.* compact, lacking ovarian features and with a single sperm duct), while testis morphology in secondary males includes an ovarian-like lumen, lamellar structure and sperm sinuses in the gonad wall (Reinboth, 1962, 1970; Smith, 1975; Robertson and Warner, 1978; Warner and Robertson, 1978; Robertson *et al.*, 1982; Shapiro and Rasotto, 1993).

It is not always feasible, however, to identify both possible development pathways in males using only morphological analysis of the testes. For example, in certain protogynous labrids, serranids and epinephelines, the testes of some specimens can exhibit a morphology characteristic of secondary males without necessarily having passed through a functional female phase (Sadovy de Mitcheson and Liu, 2008). In species such as humphead wrasse *Cheilinus undulatus* Rüppell, 1835, threadfin wrasse *Cirrhilabrus temminckii* Bleeker, 1853 and camouflage grouper *Epinephelus polyphekadion* (Bleeker, 1849), some males can develop directly from an initial juvenile female phase, retaining the ovarian-like lumen and lamellar structure in their testes (Kobayashi and Suzuki, 1990; Rhodes and Sadovy, 2002; Sadovy *et al.*, 2003). In the chocolate hind *Cephalopholis boenak* (Bloch, 1790), all undifferentiated juveniles pass through an ovarian phase and then an inactive bisexual phase characterized by the presence of a lumen, oocytes in primary growth and scattered inactive sperm tissue in gonads (Liu and Sadovy, 2004). It is from this inactive bisexual phase that individuals ultimately differentiate into functional females or males. The latter preserve the lumen and a lamellar structure in the testis while developing sperm sinuses in the gonad wall, making them morphologically identical to the testes of males produced by sexual inversion of functional females. In the catface rock cod *Epinephelus andersoni* Boulenger, 1903, the gonads of all individuals commence ontogeny as immature females, but before first sexual maturation, some begin to develop as males, producing small inactive bisexual individuals (Fennessy and Sadovy, 2002). Some of these small inactive bisexual individuals may then differentiate directly into immature males and thereafter into mature males, while others may develop into large inactive bisexual individuals and then into mature males. In both development pathways, the testes of mature males exhibit an ovarian-like lumen and a lamellar structure without having passed through a functional female phase. Males that develop directly from a juvenile female or an inactive bisexual phase are termed prematurational secondary males (these are considered functional analogues of primary males but are not homologous to them), while those produced by sexual inversion are called postmaturational secondary males (Robertson and Warner, 1978; Warner and Robertson, 1978; Robertson *et al.*, 1982; Pandian, 2011). This is why the distinction between monandric and diandric species is complicated and requires more conclusive criteria. The presence in a population of inactive males and/or inactive bisexual individuals smaller than the minimum size of sexual maturity is therefore an additional criterion to be considered when determining if a species is monandric or diandric.

The largest labrid in the western Atlantic, hogfish *Lachnolaimus maximus* (Walbaum, 1792) is distributed from North Carolina to the northeast coast of South America, including Bermuda, the entire Gulf of Mexico and the Caribbean Sea (Westneat, 2002). Sought after for its excellent taste, it is a target species in Florida recreational and commercial fleets (McBride *et al.*, 2001; McBride and Murphy, 2003). In the southern Gulf of Mexico, it is associated with the Mexican commercial grouper and snapper fisheries on Campeche Bank (SAGARPA, 2012). This labrid reproductive biology has been analysed for populations in Florida (Davis, 1976; McBride and Johnson, 2007), Cuba (Claro *et al.*, 1989) and Puerto Rico (Colin, 1982). In these regions, spawning in *L. maximus* is seasonal, prolonged and frequent (from autumn to spring). It is a protogynous hermaphrodite with pronounced sexual dichromatism; its initial colour phase is characteristic of females and the terminal phase of males. In these studies, hogfish has been characterized as a monandric species, on the assumption that all analysed males must be functionally secondary because their testes contained a lumen, and consequently, that the sex of individuals had changed from functional female to male. However, labrids exhibit a diverse range of sexual patterns and caution is needed when applying testicular morphology or body colour as an indicator of reproductive function. Labrid testicular morphology does not always coincide with the male differentiation process (Sadovy de Mitcheson and Liu, 2008). Indeed, prematurational sex change appears to be common in parrotfishes (Sparisomatinae; Robertson and Warner, 1978) and can occur in wrasses (*e.g. C. undulatus*, Sadovy *et al.*, 2003; and *Halichoeres radiatus* (Linnaeus, 1758), Warner and Robertson, 1978), which allows the supposition that some hogfish secondary males could be of prematurational origin and the species could therefore be diandric. To test this hypothesis, a detailed histological study was done of gonad specimens from a *L. maximus* population from Campeche Bank in the southern Gulf of Mexico to detect the presence of any immature individuals in the inactive bisexual stage and/or of small inactive males.

MATERIALS AND METHODS

Lachnolaimus maximus specimens were collected along the north coast of the Yucatan Peninsula near the ports of Celestún (20°52'N, 88°45'W), Dzilam de Bravo (21°30'N, 88°45'W) and Río Lagartos (21°40'N, 88°10'W) in the state of Yucatan, Mexico. In June 2011 and monthly from July 2013 to August 2014, professional fishers used harpoons to collect specimens (N = 1,975) during daylight hours (0700-1800 hours) on bottoms at 6 to 22 m depth. All specimens were measured (fork length, FL; 0.1 cm) and weighed (whole and gutted weights, WW and GW; 1 g), and their gonads weighed (gW; 0.1 g) to calculate gonadosomatic index values [GSI = 100 × (gW/GW)]. The colour phase of each specimen was recorded. Heterogeneity in oocyte distribution has been observed in the right ovary lobe, therefore only a fragment of the middle portion of the left ovary lobe of each specimen was collected (Nóh-Quiñones *et al.*, 2017) and fixed in Bouin's solution. For males, a single sample from the middle of any testis lobe was collected (McBride and Johnson, 2007) and fixed in Carnoy's solution. When possible, individual sex was inferred from apparent colouring phase. After dehydration in 90% and 100% alcohols, and Paraplast® impregnation and inclusion, 6 µm sections were cut from the gonads. Gonads from females were stained using Martoja's one-step trichrome stain and those from males with the Feulgen-Rossenbeck reaction (Gabe, 1968).

Using a microscope, individuals were visually identified as female, transitional individual or male based on established criteria (Sadovy and Shapiro, 1987). When no clear classification was possible, the possibility of "bisexual" immatures was analysed considering previous observations of the diandric groupers *E. andersoni* and *C. boenak* (Fennessy and Sadovy, 2002; Liu and Sadovy, 2004). All females and males were placed into a reproductive phase according to their sexual development based on criteria proposed by Brown-Peterson *et al.* (2011): immature, developing (including early developing subphase), spawning capable (including actively spawning and past-spawner subphases for females), regressing, and regenerating. Individuals classified as developing, spawning capable, regressing and regressed were considered mature (*i.e.* adult).

To analyse the effect of the type of protogynous hermaphroditism on population structure, comparisons were done of the individuals in each sex category (including "bisexuals") for average size, using a Kruskal-Wallis nonparametric test, and for size frequency distribution, using a Kolmogorov-Smirnov nonparametric test. Overall sex proportion was calculated as the number of females per male (F:M), excluding transitional individuals and "bisexuals". The resulting value was analysed with a Pearson χ^2 test to determine if it was near a balanced sex ratio. All statistical analyses were run with the INFOSTAT package (Di Rienzo *et al.*, 2018) using a $\alpha = 0.05$.

RESULTS

Histological analysis showed that 70% of the examined *L. maximus* gonads were from females and 25% from males. Of the females, 8% were immature and 92% mature (Tab. I). All ovaries exhibited a lumen and ovarian lamellae with oocytes in different developmental stages in the stroma.

Table I. – Sex and maturity status; number of individuals; gonadosomatic index (GSI) range; mean (± standard deviation) and median length (fork length, FL); size and weight (whole weight, WW) ranges of *Lachnolaimus maximus* collected in June 2011 and from July 2013 to August 2014 on Campeche Bank.

Sex	Maturity status	N	GSI (%)	Length (cm FL)			Weight range (g WW)
				Mean (± SD)	Median	Range	
Female	Immature	113	0.05-0.88	22.7 (4.4)	24.0	10.3-31.2	25-645
	Mature	1,273	0.04-10.38	26.8 (3.7)	26.9	13.9-39.4	91-1,132
	Total	1,386	0.04-10.38	26.5 (3.9)	26.7	10.3-39.4	25-1,132
"Bisexual"	–	20	0.13-0.82	28.6 (2.5)	28.6	24.8-33.5	311-777
Transitional	–	73	0.05-1.16	30.4 (3.0)	30.2	24.6-37.6	321-951
Male	Mature	496	0.02-1.35	33.2 (3.7)	32.8	24.5-47.4	299-1,885

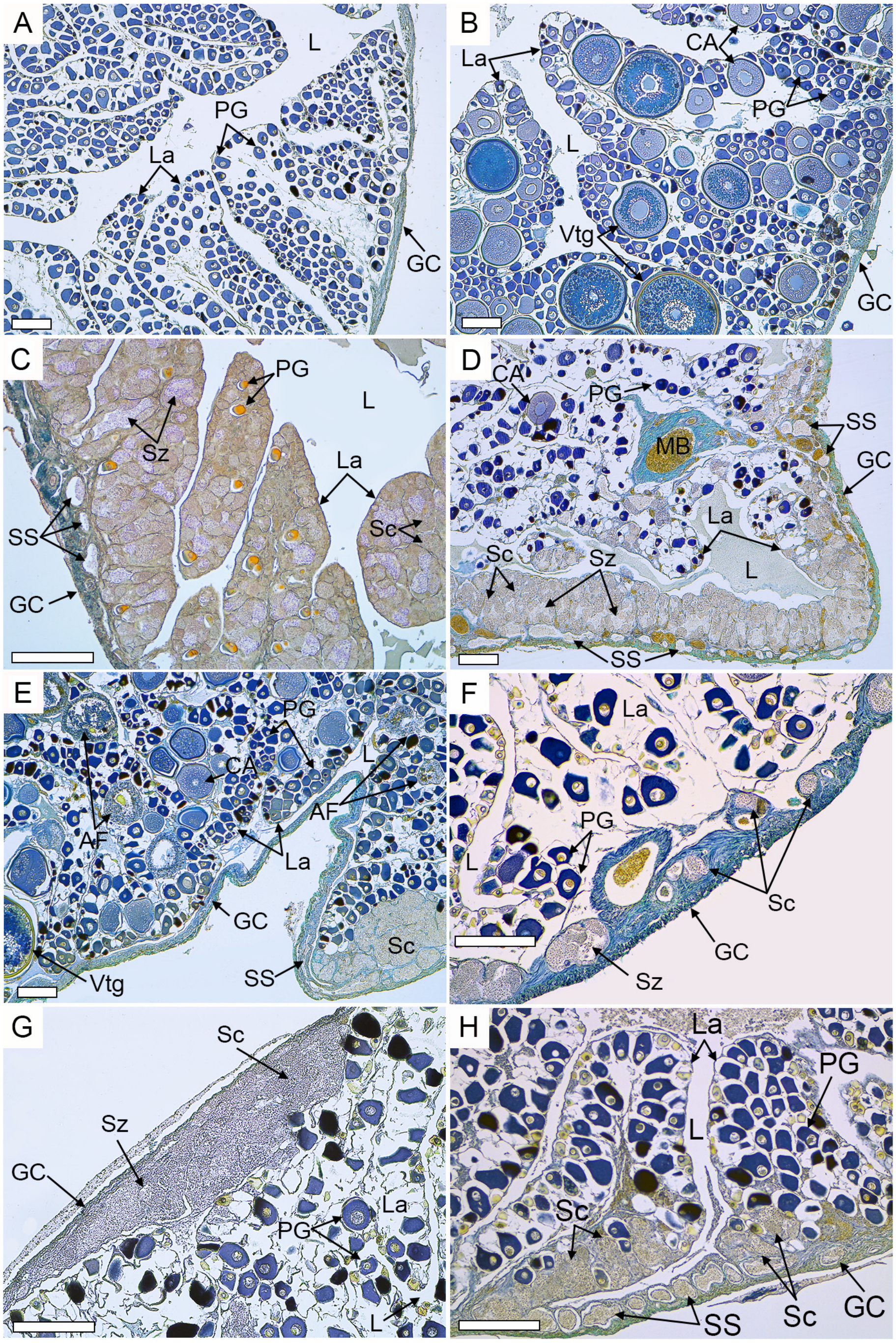

Figure 1. – Transverse sections of *Lachnolaimus maximus* gonads from Campeche Bank. **A**: Immature female (27.9 cm FL), collected October 2013 at Dzilam de Bravo; **B**: Mature active female in spawning capable reproductive phase (27.8 cm FL), collected October 2013 at Celestún; **C**: Mature active male in spawning capable reproductive phase (32.4 cm FL), collected January 2014 at Río Lagartos; **D**: Transitional (33.0 cm FL), collected January 2014 at Celestún; **E**: Transitional (26.4 cm FL), collected March 2014 at Río Lagartos; **F**: "Bisexual" (26.3 cm FL) collected June 2014 at Dzilam de Bravo; **G**: "Bisexual" (28.3 cm FL), collected January 2014 at Río Lagartos; **H**: "Bisexual" (25.0 cm FL) collected October 2013 at Río Lagartos. AF: atretic follicle; CA: cortical alveolar oocyte; GC: gonad capsule; L: lumen; La: lamellae; MB: muscle bundle; PG: primary growth oocyte; Sc: spermatocyte; SS: sperm sinus; Sz: spermatozoa; Vtg: vitellogenic oocyte. Gabe and Martoja's one-step trichrome stain (A, B, D, E, F, G and H); Feulgen-Rossenbeck reaction (C). Scale bars = 100 µm.

Immature females only exhibited oocytes in primary growth stages in their ovaries (Fig. 1A), while in mature females the ovaries contained oocytes in different stages of development, and even some atretic follicles and muscle bundles, depending on their reproductive phase (Fig. 1B; Tab. II). All males were mature and most exhibited an internal testicular morphology typical of postmaturational secondary males: a membrane-lined central cavity and numerous testicular lobules enclosed in a lamellar structure (92%), and sperm sinuses in the gonad wall (58%). Twelve percent of the males exhibited some remnant primary growth oocytes in the testes (Fig. 1C; Tab. II).

The remaining 5% of the examined gonads (N = 93) were ovotestes characterized by a typically ovarian internal structure (*i.e.* lumen and lamellae) and the presence of ovarian and testicular germinal tissues (Fig. 1D-H). The ovarian germinal tissue, which predominated in the lamellae of all these ovotestes, was inactive (*i.e.* presence of primary

Table II. – Histological features of immature and mature female, "bisexual", transitional and male *Lachnolaimus maximus* gonads collected on Campeche Bank. PG: primary growth oocyte; SG: secondary growth oocyte; CA: cortical alveolar oocyte; Vtg: vitellogenic oocyte; OM: oocyte maturation; AF: atretic follicle; MB: muscle bundle; L: lumen; SS: sperm sinus. +: observed; –: not observed; (+): observed in most individuals; [+]: observed in few individuals. ^{1}: spermatocytes, spermatids and spermatozoa; occurrence and proportion of SG and MB in ovaries depend of female reproductive phase.

Sex	Maturity status	Ovarian tissue					Testicular tissue[1]			MB	Gonadal structure	
		PG	SG				Within gonad capsule	Below gonad capsule	Within lamellae		L	SS
			CA	Vtg	OM	AF						
Female	Immature	+	–	–	–	–	–	–	–	–	+	–
	Mature	+	+	+	+	+	–	–	–	+	+	–
"Bisexual"	–	+	[+]	–	–	–	[+]	+	–	–	+	[+]
Transitional	–	+	[+]	[+]	–	[+]	[+]	(+)	[+]	(+)	(+)	[+]
Male	Mature	[+]	–	–	–	–	–	–	+	[+]	(+)	(+)

Table III. – Monthly number of sample fish and monthly percent of female and male classified according to reproductive phases for *Lachnolaimus maximus* collected in June 2011 and from July 2013 to August 2014 on Campeche Bank. I: immature; D: developing (with early developing subphase included); SC: spawning capable (with actively spawning and past-spawner subphases included for females); R_{egr}: regressing; and R_{ege}: regenerating.

	Female						"Bisexual"	Transitional	Male				
Month	N	% I	% D	% SC	% R_{egr}	% R_{ege}	N	N	N	% D	% SC	% R_{egr}	% R_{ege}
January	83	8	22	48	19	3	3	6	59	7	93	0	0
February	109	2	12	76	5	5	1	4	74	7	93	0	0
March	85	1	6	89	2	2	0	1	34	9	91	0	0
April	119	0	0	84	15	1	0	0	48	8	92	0	0
May	136	2	4	60	23	11	1	0	35	11	86	0	3
June	141	19	10	21	42	8	3	13	45	5	93	0	2
July	206	9	2	11	75	3	0	2	7	14	72	0	14
August	139	15	4	10	71	0	3	3	25	24	68	4	4
September	117	17	3	0	80	0	0	3	9	0	100	0	0
October	105	11	41	4	44	0	5	10	27	4	96	0	0
November	77	3	19	60	14	4	1	12	40	0	100	0	0
December	69	3	45	29	16	7	3	19	93	1	98	0	1
Total	1386						20	73	496				

growth oocytes, and occasionally cortical alveolar or vitellogenic oocytes). In contrast, the testicular germinal tissue was active (*i.e.* presence of spermatocytes, spermatids and spermatozoa) but not abundant. It was topographically separate from the ovarian tissue since it was confined in lobules grouped in lamellae located underneath the gonad wall and/or in crypts inside the gonad wall. Sperm sinuses were found in the gonad wall in some of these ovotestes. These individuals were not considered active males due to the scarcity of testicular tissue development in the ovotestes. Most (79%; N = 73) individuals exhibited sparse distribution of remnant structures in the lamellae (*i.e.* atretic vitellogenic stage oocytes and/or muscle bundles). These are typical of previous ovarian activity and the individuals were thus considered as undergoing sexual inversion and classified as transitional (Fig. 1D, E; Tabs I, II). No structures of this sort were observed in the ovotestes of the remaining individuals (21%; N = 20), which were consequently considered as "bisexual" individuals with a tendency for testicular tissue proliferation in the ovotestes (Fig. 1F-H; Tabs I, II). Sixteen of these specimens exhibited the initial coloration phase typical of females, two had the terminal coloration typical of males and the remaining two were in an undefined coloration phase. Individuals with "bisexual" gonads were collected during eight months of the annual cycle, when mature females and males were in spawning capable phase and transitional individuals were present in collections (Tab. III). The GSI values for "bisexual" and transitional individuals and immature females were low and their ranges were similar (Tab. I).

Average size (Kruskal-Wallis test) and size frequency distribution (Kolmogorov-Smirnov test) differed between sexual categories, except between transitional and "bisexual" individuals (Tabs IV, V; Fig. 2). Minimum sizes for the "bisexual", male and transitional specimens were very similar (24.5-24.8 cm FL), and their size ranges partially overlapped. The size range of "bisexual" individuals also

Table IV. – Kruskal-Wallis test results for mean sizes (fork length, cm) by sexual categories of *Lachnolaimus maximus* from Campeche Bank. SD: standard deviation; d.f.: degrees of freedom; *H*: H statistic; *P*: probability value. Mean values for fork length with a letter in common are not significantly different.

Sexual categories	N	Mean	SD	Rank	d.f.	*H*	*P*
Female	1,386	26.4^{a}	3.9	761.37	3	765.45	< 0.0001
“Bisexual”	20	28.6^{b}	2.5	1046.78			
Transitional	73	30.4^{b}	3.0	1279.7			
Male	496	33.2^{c}	3.7	1575.97			

Table V. – Kolmogorov-Smirnov test results for size-frequency distributions (fork length, cm) by sexual categories of *Lachnolaimus maximus* from Campeche Bank. *KS*: KS statistic; *P*: probability value. *: not significantly different.

Sexual categories	N	*KS*	*P*
Female/“Bisexual”	1,386/20	0.31	< 0.05
Female/Transitional	1,386/73	0.45	< 0.01
Female/Male	1,386/496	0.67	< 0.01
Transitional/”Bisexual”	73/20	0.28	< 0.20*
Male/“Bisexual”	496/20	0.56	< 0.01
Male/Transitional	496/73	0.36	< 0.01

partially overlapped with that of immature females (Tab. I; Fig. 2). All “bisexual”, male and transitional individuals had a size greater than that of the minimum size at first maturity for this species (13.9 cm FL) (Tab. I). The overall sex proportion (2.79:1.00) was biased in favour of females (χ^2 de Pearson = 420.88; df = 1; $P < 0.0001$).

DISCUSSION

Twenty *L. maximus* specimens from Campeche Bank (1% of all fish examined) could not be unequivocally classified, based on established criteria (Sadovy and Shapiro, 1987), into one of the three sexual categories characteristic of a protogynous sequential hermaphrodite species (female, transitional individual or male). Because they simultaneously exhibited both ovarian and testicular tissues, although at different abundances and activity levels, these individuals were considered “bisexual”. Germinal tissue configuration in the “bisexual” gonads and the transitional gonads (unmixed ovarian and testicular tissues) falls within the undelimited type 1 category rather than the undelimited type 2 generally observed in labrids (Sadovy and Shapiro, 1987; Sadovy de Mitcheson and Liu, 2008). This germinal tissue configuration is very similar to that observed by Kobayashi and Suzuki (1990) in the gonads of an individual of the monandric protogynous labrid *C. temminckii*. The “bisexual” gonads of *L. maximus* also had certain morphological similarities with *E. andersoni* gonads from small and large inactive bisexu-

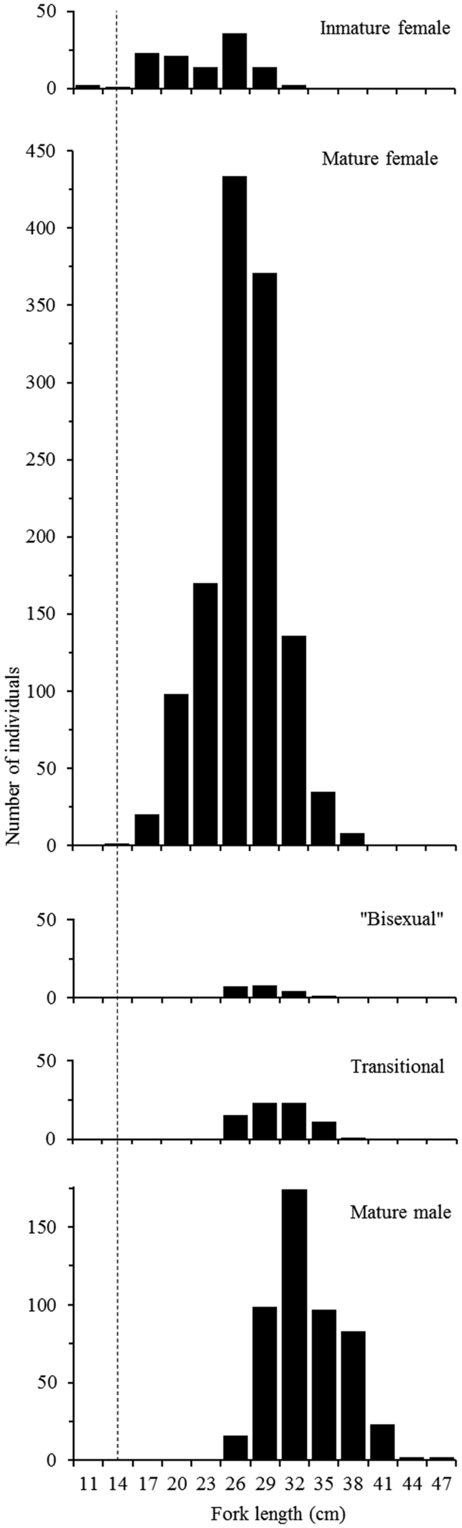

Figure 2. – Size-frequency distributions of immature and mature female, “bisexual”, transitional and mature male of *Lachnolaimus maximus* collected in June 2011 and from July 2013 to August 2014 on Campeche Bank. The dashed vertical line represents the minimum size at which females became sexually mature for the first time.

als (sBI and LBI) (Fennessy and Sadovy, 2002), and *C. boenak* gonads in bisexual phases Bi-1, Bi-2 and Bi-5 (Liu and Sadovy, 2004). However, unlike the bisexual *C. boenak* individuals, the "bisexual" *L. maximus* individuals evaluated here did not exhibit proliferation of testicular tissue within the ovarian stroma, near the periphery of the lamellae.

In contrast to *E. andersoni* and *C. boenak*, no small inactive bisexuals (sBI) or individuals with Bi-1 or Bi-2 gonads of a length smaller than the minimum size at first maturity were observed in the examined *L. maximus* specimens. Considering the schematic of possible gonad ontogeny proposed by Fennessy and Sadovy (2002) to illustrate diandric protogyny in *E. andersoni*, it is likely that all the mature-sized "bisexual" *L. maximus* specimens observed in the present study could have arisen from large inactive females (LFI) (Fig. 3). In protogynous species, some mature females (FM) can regress to an inactive stage without preserving visible traces of recent ovarian activity in the gonads (*i.e.* atretic vitellogenic oocytes and/or muscle bundles). They can ripen and regress again several times, before undergoing sex transition from functional female to postmaturational male (pathway a). Three additional developmental pathways involving LFI may explain the presence of the "bisexual" specimens in *L. maximus* (Fig. 3). In pathway b, the "bisexuals" may have originated from regressing LFI that had spawned previously at least once. Differentiation of scarce active testicular tissue in their ovaries could indicate the beginning of sex transition from functional female to male. Consequently, the "bisexuals" observed here would be atypical transitional individuals. This would explain the similarity in size ranges and time occurrence between supposedly "bisexual" specimens and distinctive transitional individuals. All secondary males in this scenario would be postmaturational and the species would be monandric. For pathway c, the "bisexual" specimens may originate from LFI still in the immature phase due to delayed sexual maturity. The development of testicular crypts or lobules in their ovaries would be a precocious and ephemeral process prior to their initial reproduction as females. Once they reached first sexual maturity, these individuals would continue to develop as functional females before transforming into functional males through sex inversion. For example, Liu and Sadovy (2004) identified scattered testicular tissue in the ovaries of immature Bi-2 *C. boenak* females. In this case, the *L. maximus* "bisexuals" would be immature females larger than the minimum size at first primary maturity. Presence of a size range overlap between the "bisexuals" and a portion of immature females would support this interpretation. As in the preceding case, secondary males would all be postmaturational and the species would be monandric. In pathway d, the "bisexual" specimens may originate from LFI in a delayed immature phase. But during their somatic growth, development of testicular tissue in their ovaries would be slow and steady. Before reaching first maturity as females, they would transform directly into active males without passing through a functional female phase. These "bisexuals" would be analogous to the large inactive bisexual (LBI) specimens observed in *E. andersoni* (Fennessy and Sadovy, 2002). Secondary males would therefore be of premeturational or postmaturational origin, making the species diandric.

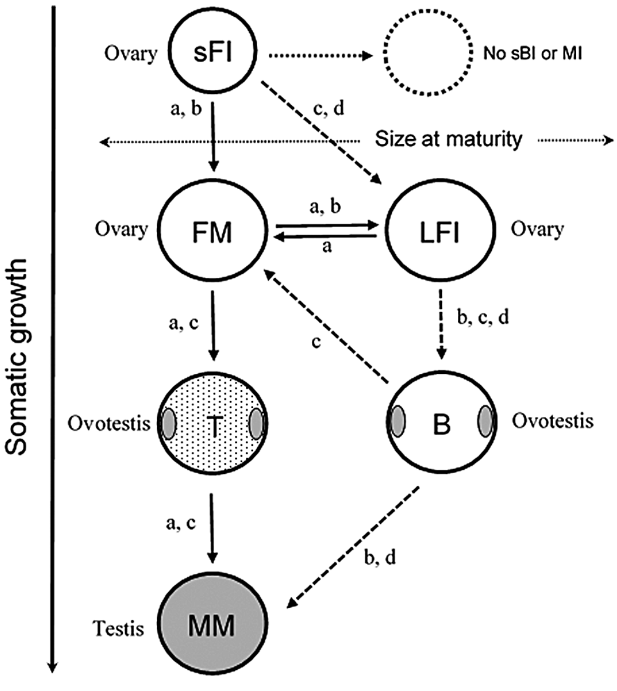

Figure 3. – Hypothetical scheme of sexual development in the protogynous *Lachnolaimus maximus*. sFI: small inactive female (*i.e.* immature); sBI: small inactive bisexual; MI: immature male; FM: mature active female; LFI: large inactive female (*i.e.* immature or mature inactive); T: transitional; B: "bisexual" (ovotestes with prevailing inactive ovarian tissue and scarce active testicular tissue); MM: mature active male. White colour represents female tissue; grey colour represents male tissue and stippled pattern represents remains of prior spawning as a female. Solid arrows represent known developmental pathways and dashed arrows hypothetical development pathways. a, b and c: postmaturational secondary male pathways; d: prematurational secondary male pathway (modified after Fennessy and Sadovy, 2002).

Only postmaturational sex change has been reported for *L. maximus* from Florida (Davis, 1976; McBride and Johnson, 2007) or Cuba (Claro *et al.*, 1989). "Bisexual" specimens may not have been observed in these previous studies for at least two reasons. First, they appear to be relatively uncommon in field collections, meaning sample size would have been too small to detect them: for example, 24 gonad histological preparations were analysed by Davis (1976) and 465 by Claro *et al.* (1989). Second, even when using a large gonad histological section sample size (N = 1,662; McBride and Johnson, 2007), "bisexuals" may have been confounded with transitional individuals or the discrete islets of testicular tissue appearing in the gonads of juvenile females may not have been identified.

The possibility that *L. maximus* exhibits diandric hermaphroditism is by no means unequivocally proven and remains hypothetical. The characteristics of the Campeche Bank *L. maximus* population do not coincide with the hypothesis of diandric development. Indeed, the size by sex structure (bimodal with larger males than females) and the overall sex proportion (more females than males) were typical of a monandric protogynous species. This differs from diandric populations in which male and female size ranges can overlap completely and sex proportion approaches unity (Sadovy and Shapiro, 1987). However, the characteristics of diandric populations depend closely on their proportion of primary males. A small number of primary males does not significantly alter a bimodal distribution of size versus sex and the bias in favour of females would remain (Sadovy and Shapiro, 1987). In this sense, if all the "bisexual" *L. maximus* specimens reported here are considered as prematurational males (*i.e.* functional analogues of primary males), they would represent barely 1% of the analysed individuals and only 4% of secondary males. The principal argument for rejecting the diandric hypothesis for *L. maximus* is that the "bisexual" gonads identified in the present study could be those of atypical transitional individuals. Indeed, "bisexual" and typical transitional individuals exhibited the same size-distribution pattern, were larger than minimum size at first maturation in this species and appeared simultaneously through time (*i.e.* in collections done throughout an annual cycle). Histological examination of the gonads of a larger sample of individuals smaller than those analysed here would help to determine if sBI or small inactive males are present in this *L. maximus* population and to confirm this species' sexual pattern.

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